THE ETIOLOGY OF PORAL CLOSURE

II. The Role of Staphylococcal Infection in Miliaria Rubra and Bullous Impetigo

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(a) *Observations on infection in natural miliaria*

Although lipoid depletion afforded a promising field of study in my earlier work on miliaria (2), the thought was always present that bacterial infection might eventually prove to be of decisive significance. However, it was not until 1945 in the Moluccas that an opportunity arose to investigate the part played by pathogenic bacteria in miliaria. Unfortunately the program was hampered by the military changes that followed the end of hostilities; yet, despite many imperfections, enough information was gathered to indicate that the infective hypothesis must be seriously considered.

As an initial point, miliaria resembles tropical bullous impetigo (pyosis mansoni or "tropical pemphigus")* in its pathology and some other characteristics. Both are obstructive, vesicular diseases affecting the orifices of sweat glands (27). They often concur in the same patient though this in itself may not be relevant. Their clinical and pathologic differences could possibly be accounted for by the fact that bullous impetigo chiefly affects the apocrine pores whereas miliaria rubra affects the eccrine pores. Although perhaps not the only factor, most workers agree (5) that bullous impetigo is due principally to an infection of the apocrine pores by bacteria, especially by *Staphylococcus aureus*. So close are the analogies that it might be well argued that miliaria is of a similar causation.

Another pointer to infection in miliaria is the frequent clinical association of miliaria with staphylococcal impetigo ("pemphigus") neonatorum (6) and with furunculosis. The observation that antiseptics are beneficial in miliaria (28) also supports the concept of infection.

To turn to the sphere of histology, it is generally true that neutrophilic infiltration indicates bacterial invasion. By this rule, miliarial lesions, even when fully developed, usually show but little evidence of the presence and effects of bacteria; in fact lymphocytes far outnumber neutrophils in the exudate (2, 9). Only occasionally are pustular lesions encountered; for instance, Plate 3 gives an early, and Plate 4 a mature pustule. When dealing with lesions as frankly infected as these it is quite impossible to make the fundamental distinction between primary and secondary infection. The distinction is perhaps easier when attention is focussed on the very earliest lesions within keratin rings. For it seems that the keratin rings of the sweat pores merit the same intensive study in relation to this problem of infection as they do in relation to the effects of lipoid depletion (2).

* Since bullous impetigo is not a true pemphigus, the name "tropical pemphigus" is a misnomer.

The normal anatomy of the keratin ring is illustrated by the cross section in Plate 1, more details of its relations being given in Plate 2 where it can be seen that the ring, appearing dark in the photograph, dips down to surround a considerable length of the terminal portion of the sweat duct lumen. In my experience, micro-organisms are not seen or are scanty or difficult to recognize in

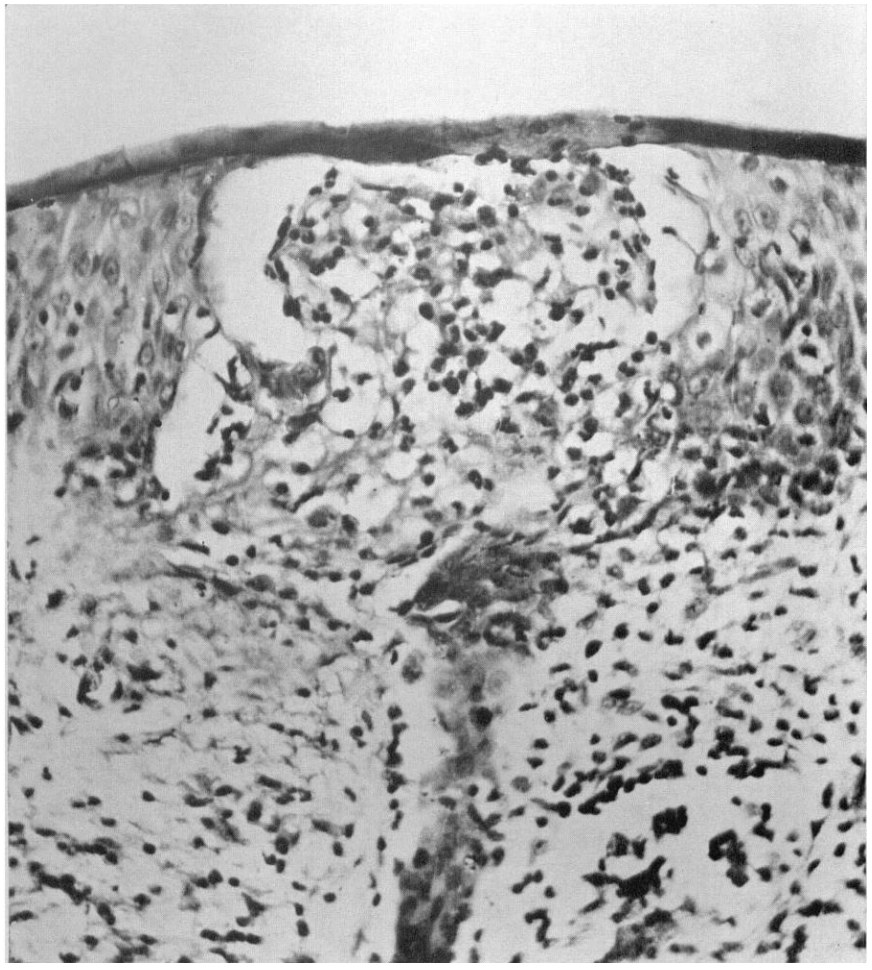


PLATE 3. A miliarial vesicle showing slight but definite evidence of infection. There are fairly numerous neutrophils in the exudate. (Haematoxylin and Biebrich Scarlet. × 400.)

or near normal pores. This holds true even though it has been shown (19, 20, 21, 22) that an abundant flora resides on and within the superficial layers of normal skin.

By contrast, Plates 5 to 9 show that there is an intense bacterial colonization of the pores in the earliest lesions of tropical miliaria. All these illustrations are from patients whose biopsies also contain typical fully-developed miliarial lesions.

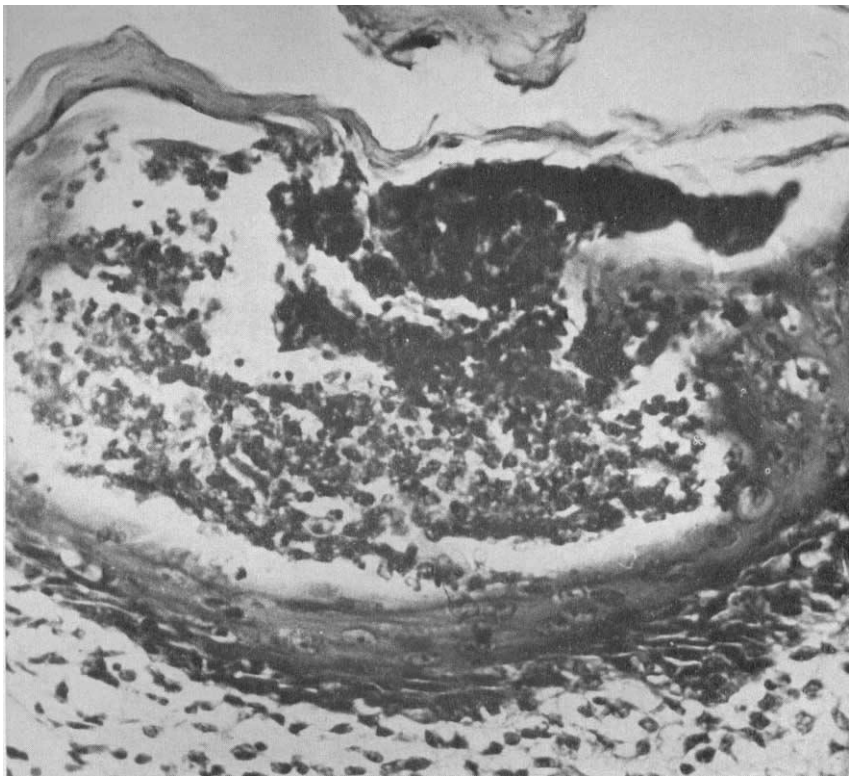


PLATE 4. A purulent miliarial vesicle illustrating frank infection. (The associated pore, just outside the upper border of the picture, contains numerous apparent cocci.) (H. and B. S. × 400.)

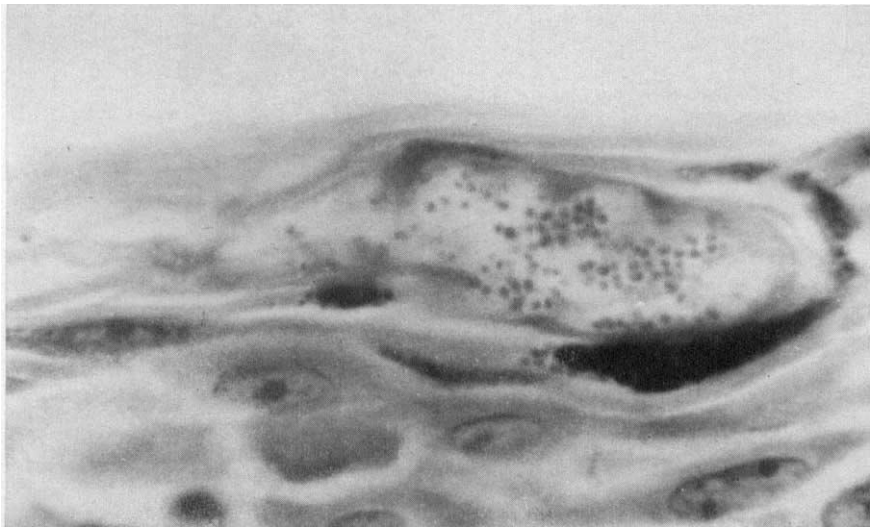


PLATE 5. Clumps of apparent cocci in a pore from skin affected by miliaria. This may represent the first lesion of the disease. See Text. (H. and B. S. × 1800.)

PLATE 6. A section showing apparent cocci in a pore from skin affected by miliaria. An arrow indicates the area where the lumen appears to be occluded. (H. and B. S. × 1800.)

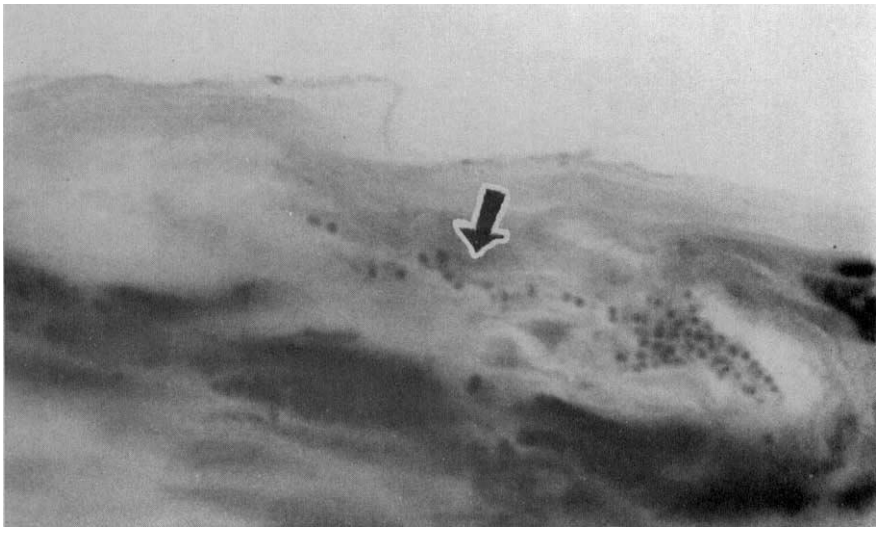


PLATE 7. The pore of Plate 6 in an adjacent section. The lumen still appears to be occluded. (H. and B. S. × 1800.)

Plate 5 illustrates a keratin ring whose lumen contains a large number of cocci, some occuring in clusters. Similar organisms in similar lesions from the same biopsy stain positively by Kirkpatrick's (29) Gram method and it is diffi-

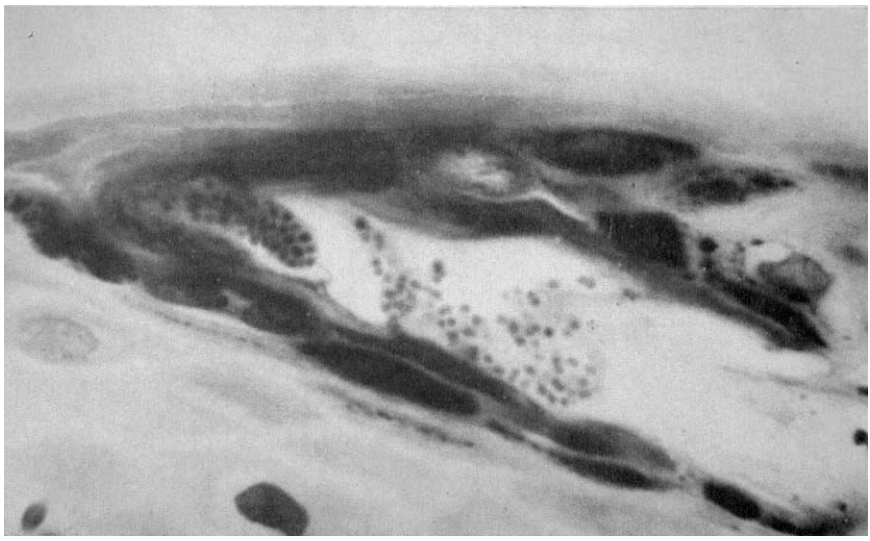


PLATE 8. Apparent cocci in a dilated (apparently obstructed) duct in miliaria. (H. and B. S. × 1800.)

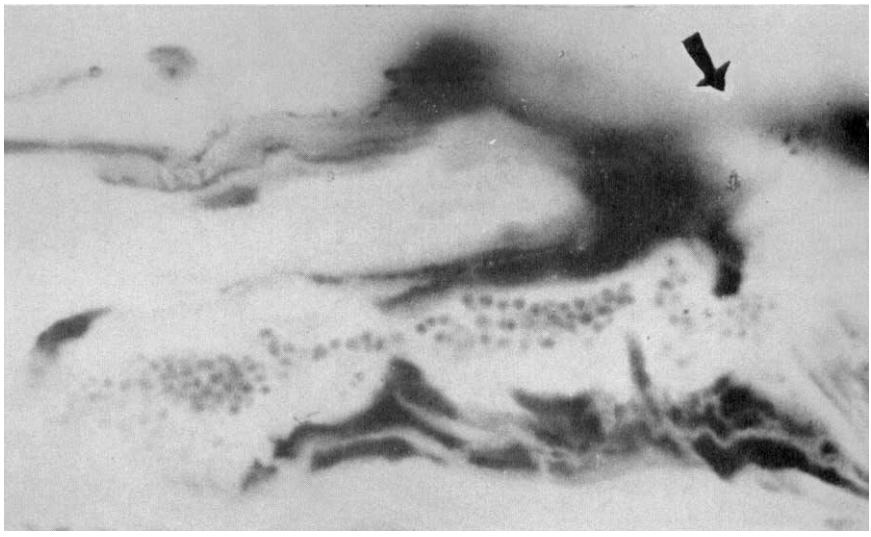


PLATE 9. Numerous Gram-positive cocci in the keratin ring of a pore. The opening of the pore is indicated by an arrow. From skin affected by miliaria. (Gram. × 1800.)

cult to deny the strong suspicion that they are staphylococci.* Plates 6 and 7 are photographs of two adjacent serial sections of another lesion from the same biopsy as the preceding section. The coccal infection of the ring appears beyond

* Henceforth in this paper when bacteria seen in sections are called staphylococci the necessary reservations as to true identity are implied.

doubt. The lumen of at least part of the keratin ring is occluded, an appearance which may be due to associated inflammatory edema or alternatively it may merely arise out of the plane of the section.

The section illustrated in Plate 8 was made from a biopsy from another subject with acute miliaria and shows numerous cocci in the superficial portion of the sweat duct. Some dilatation of the duct, apparently obstructive in nature, is noteworthy. An adjoining section in Plate 9, stained by Gram's method, shows another duct containing a large number of Gram-positive cocci.

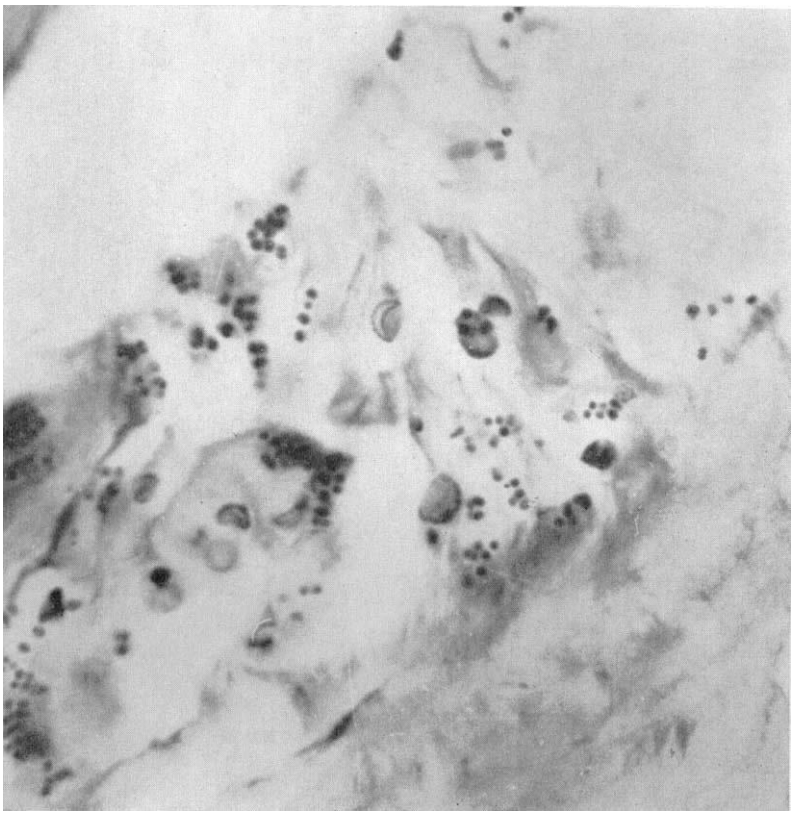


PLATE 10. Gram-positive cocci in a follicle from miliarial skin. (Gram. × 1800.)

It seems from these sections and from others not illustrated that a very significant degree of bacterial growth occurs from the beginning in precisely those pores which, being from miliarial skin, appear to show the very earliest obstructive lesions of the disease. It is therefore idle to deny that a primary causative relationship may in fact exist between obstruction of the pores and the presence of bacteria therein.

In all, 7 biopsies from different subjects affected by incipient acute miliaria have been critically examined, the total number of sections involved in this

phase of the work being about 2,000. In every subject, a considerable proportion of the pores which show early apparent obstruction are infected in the manner illustrated. Taking this group of sections as a whole, there have been found some 50 pores heavily colonized by numerous Gram-positive cocci. This proportion represents something of the order of a quarter to an eighth of the total pores present.

No cocci have been detected *within* miliarial vesciles and, as already mentioned,

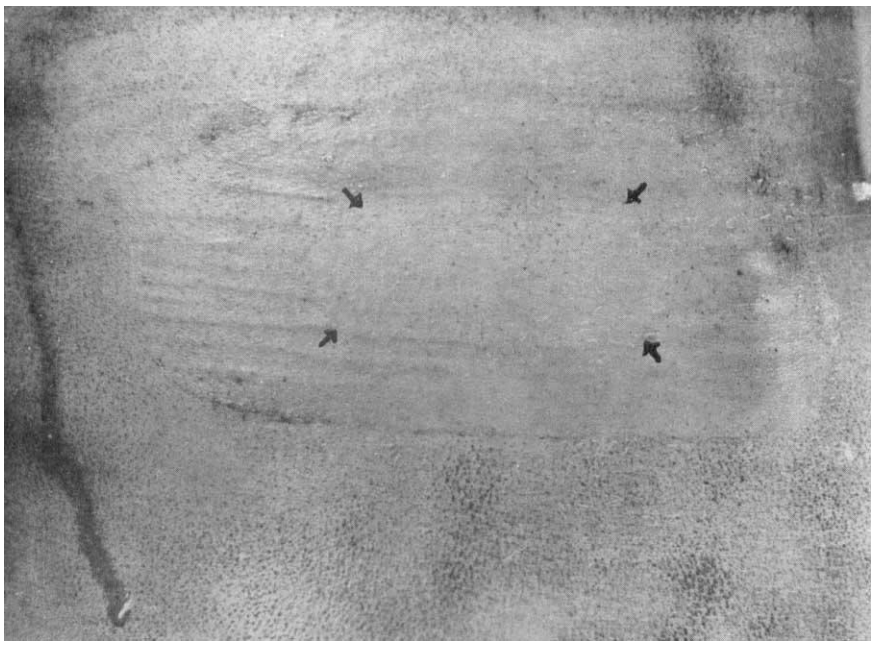


PLATE 11. A starch-iodine test demonstrates the anhidrotic effect produced by the pro longed application of a gauze pad and strapping. The small area covered by the pad is in the centre of the picture and is indicated by arrows; it contains only a few patent pores (appearing as black dots) in contrast to the normal uncovered skin near the edge of the picture. The intervening rim of skin, which was covered by strapping, is also relatively anhidrotic; it has been stripped of its hairs. No pustules were produced in this subject.

such vesicles do not as a rule show much evidence of suppuration.* It is therefore suggested that infection leads to miliaria in the following way:

(1) Bacterial growth causes obstruction of the pores.
(2) The presence of the retained sweat then causes dilatation and rupture of the ducts. The resulting vesicles are thus produced in a secondary fashion by physical means.

Why the cocci generally fail to cause frank suppuration of miliarial vesicles appears to be a problem in host-organism relationships.

Plate 8 of the earlier paper (2) is relevant here. Although not seen at the time

* In bullous impetigo, on the other hand, the vesicles regularly suppurate.

of the original description and not evident in the plate, cocci have since been identified in the keratin ring above the vesicle shown. The vesicle itself contains few if any neutrophils.

How bacteria bring about poral closure is a problem in itself. The physical bulk of clumps may be one factor; another is inflammatory edema of the keratin ring which contains them. The role, if any, of such edema cannot be determined from my material.

The clinical folliculitis so often found in miliaria has its counterpart in the tissue sections. Plate 10 shows numerous Gram-positive cocci in the depths of a follicle. The other bodies present appear to be degenerate forms of the *Pityrosporum ovale.*

(b) *The poral effects produced in the tropics by covering the skin with plain gauze pads*

My experimental work on infection in miliaria began with the application of skin scrapings from patients to normal volunteers.

Typical acute miliarial lesions were scraped and a suspension made in 0.9 per cent sodium chloride solution. Seven normal soldier volunteers who had no history of skin disease were selected and a sterile gauze pad moistened in the suspension was strapped to each. To the opposite side of the body a similar "control" pad moistened in saline alone was applied.

The subjects continued their usual duties and the pads were left undisturbed for six days. Most subjects complained of local itching under both pads; generally speaking the test side itched no more than the control. The changes observed in the skin on both sides after removal of the pads are summarized in Plate 11* and in the following analysis:

(1) The most significant observation was that the areas covered by the gauze pads showed anhidrosis on the *control* side as well as on the test or scrapings side. This bilateral absence of sweating was obvious clinically but was confirmed by the starch-iodine technic (30).

(2) The areas covered by adhesive tape were also quite dry on both sides.

(3) The areas covered by the pads were not only anhidrotic, but also showed either a crystalline (sudaminal)or a miliaria-like rash. In some subjects, there was only a suggestion of a miliarial rash, a state which might be called sub-clinical miliaria. Impetiginous pustules arose on one or both sides in five cases.

(4) The pilosebaceous follicles often appeared distended, particularly on the areas directly covered by adhesive tape.†

(5) When pustules were present, smears showed Gram-positive cocci, together

* The photographs taken of the original subjects were lost in an aircraft accident. Plate 11 is from an identical experiment since carried out in Sydney. Possibly owing to the climatic difference, the responses in Sydney were generally less constant and less complete than those obtained in the tropics.

† S. M. Peck, H. Rosenfeld, K. K. Li and A. Glick (The Mechanism of Adhesive Plaster Irritation, J. Invest. Dermat. 10: 367 [May] 1948) have given a complete review of the effects of adhesive tape on the skin.

with Gram-negative bacilli in some cases. On direct plate cultivation, a profuse growth of haemolytic coagulase-positive staphylococci was obtained. The bacilli seen in smears usually proved to be *Pseudomonas pyocyanea* (*Bacillus pyocyaneus*).

(6) The anhidrosis on the areas which were covered by the pads gradually and spontaneously disappeared within a period of 3 to 8 days. Desquamation during recovery was not conspicuous.

To summarize, the presence or otherwise of scrapings on the pads seemed to exert no effect on the outcome. The important point is that *anhidrosis was regularly produced by a saline-containing pad even in the absence of extraneous miliarial material.*

The presumption that these responses shed light upon the problem of miliaria is supported by the histologic changes now to be described.

The Histopathology of the Obstructive Anhidrosis produced by Covering the Skin: This account is based on a biopsy made from a *control* area of skin which had been rendered anhidrotic by a saline-containing pad in the manner described in the preceding paragraphs. The interval between removal of the pad and the taking of the biopsy was 24 hours. Of a total of 600 serial sections, about half were stained by haematoxylin and biebrich scarlet* and half by the Gram method.

All sweat pores in the biopsy show histologic evidence of obstruction at the level of the stratum corneum. Indeed, the changes in the pores are comparable with those described (2) as the first stage of the histopathology of natural miliaria and consist of impervious keratin rings and dilated ducts. Owing probably to the relatively short period of application of the pad (6 days), no lesions have progressed to the stage of intra-mucosal rupture of the ducts. Consequently, no deep vesicles are encountered in the sections. Their absence is not of critical importance, since vesiculation is regarded as secondary and incidental even in true miliaria.

Plate 12 typifies the general changes. The sweat duct is dilated and contains an exudate of amorphous material and, in its upper portion, some neutrophils. The keratin in the pore region contains no lumen and is *perhaps* more bulky† than normal. Little or no lymphocytic infiltration is seen in the dermis, there being here a further similarity to the first or pre-vesicular stage (2) of natural miliaria.

The obvious ballooning of the duct in Plate 13 again emphasizes that, in seeking a causal basis, attention must be focussed on the stratum corneum.

* Biebrich scarlet (Grübler, One per cent solution in buffered water at pH 6.0) has been extensively used in these studies; when differentiated by ethyl alcohol it is an excellent stain for the keratin rings of the pores (see, for instance, Plates 2, 15, 27 and 30).

† Throughout this and my earlier (2) studies the term "plug" has been reserved for a relatively large parakeratotic mass of recent origin such as is seen in late miliaria. (See) for instance, Plate 36 of Part III of this paper and also Plates 15, 16, 19 of Reference (2.),

Plate 14 shows a variation in the form of a superficial, crystalline vesicle and here again the obstruction is clearly due to changes within the keratin layer.

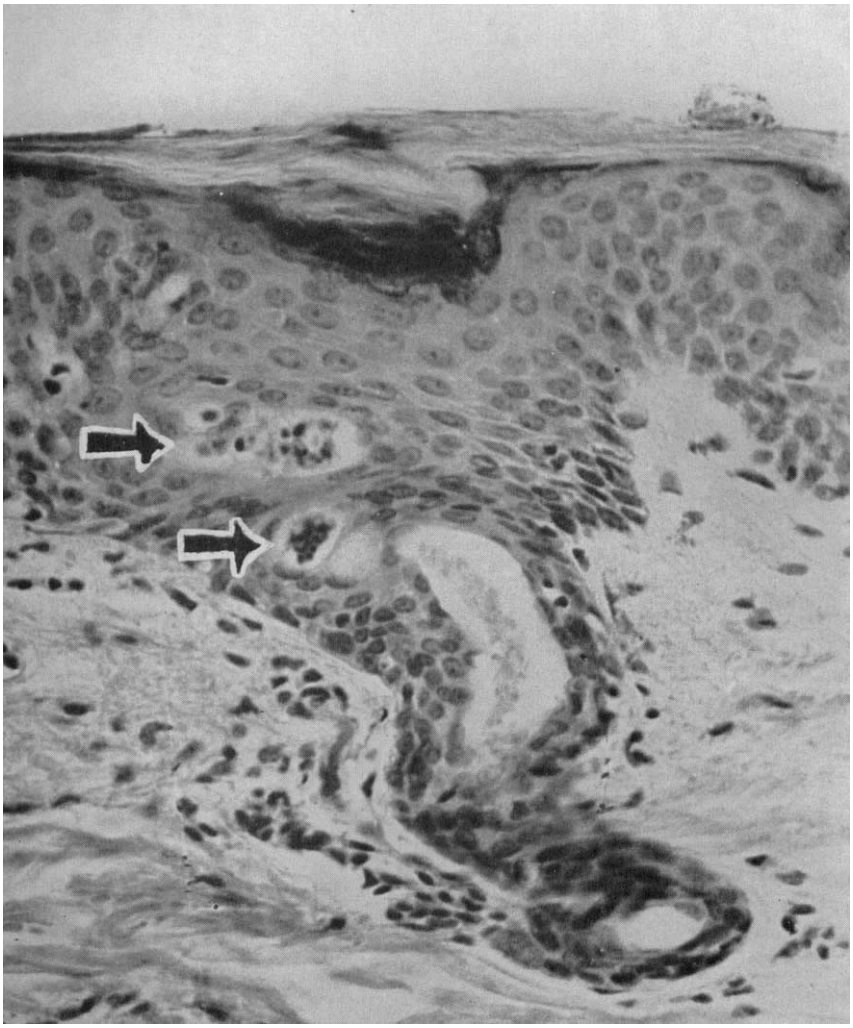


PLATE 12. A dilated duct contains granular exudate and, as indicated by arrows, groups of neutrophils. From pad-covered skin. (H. and B. S. × 530.)

Three distinct hypotheses will be tendered to explain the obstructive anhidrosis, namely (i) a local lipoid depletion (ii) edema of the stratum corneum and (iii) infection of the pores under the pad.

A fourth and less feasible hypothesis, that the responses are allergic in nature, will not be discussed.

(i) *Lipoid Depletion under the Pad:* The lipoid content of the stratum corneum probably exerts considerable influence over the patency of pores. This is best demonstrated in the anhidrotic state that follows miliaria (post-miliarial anhidrosis (2)); if a lipoid such as lanolin is smeared over a patch of post-miliarial

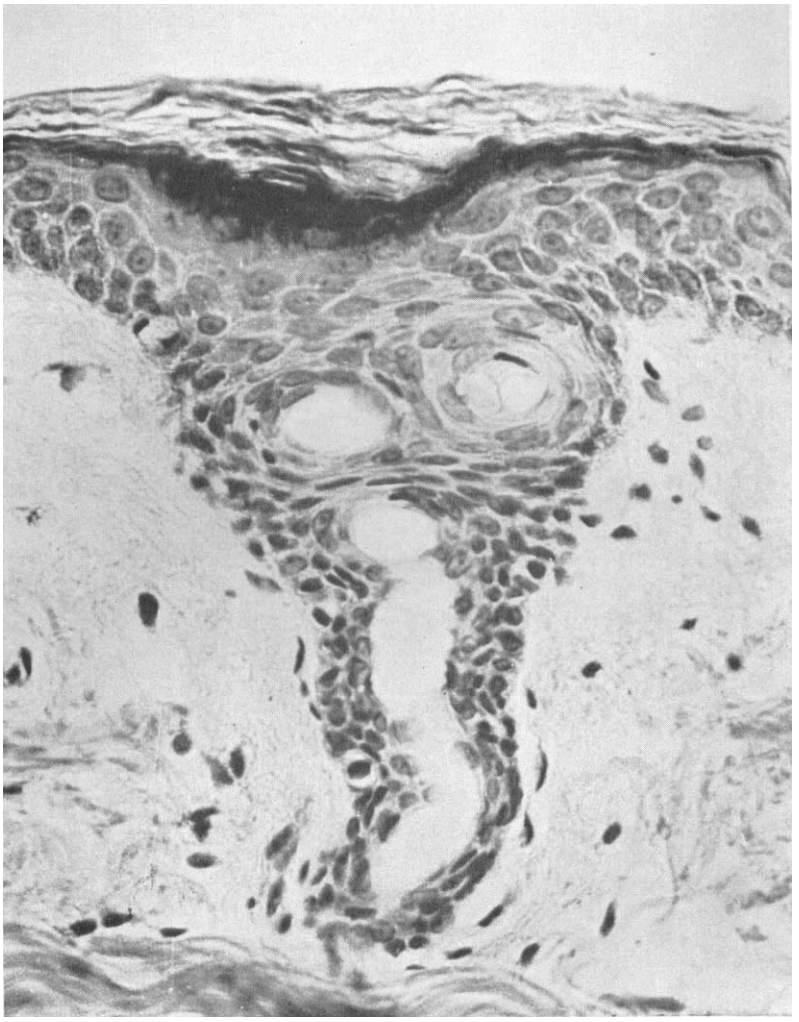


PLATE 13. A grossly dilated, probably obstructed, sweat duct from pad-covered skin. (H. and B. S. × 670.)

anhidrotic skin, an abundance of sweat thereupon reaches the surface even though large keratin plugs are present at the pores (the "lipoid response" (2)). The anhidrosis recurs when the lipoid is removed (2). It has therefore been hypothesized (2) that pores close when the lipoid content of the stratum corneum falls below a critical level.

To return to the present experiments one would hardly expect the application of a pad and strapping to bring about a lipoid depletion of the stratum corneum. Granting that the discharge of sebum continues, the protection of the covered area and its incubation* by the dressing might be expected to result in a net *increase* in the quantity of local surface lipoids.

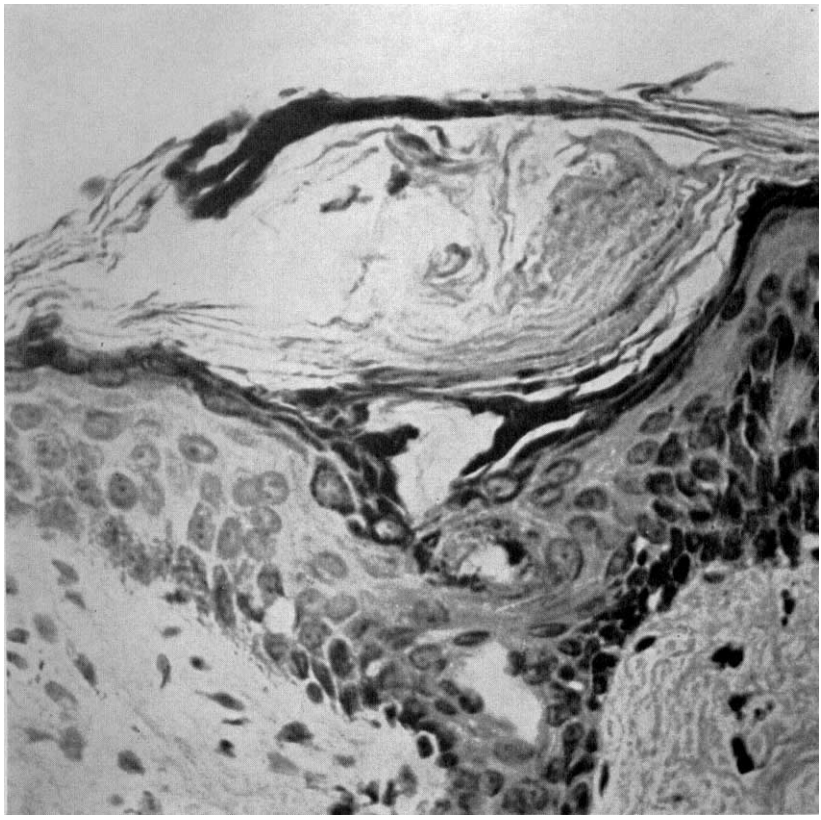


PLATE 14. A crystalline vesicle from pad-covered skin. (H. and B. S. × 670.)

As a presumptive test for net lipoid depletion, part of the anhidrotic area produced by one such experiment was smeared with lanolin on the day after the removal of the pads. Although the lanolin was left in place for five hours, there was no lipoid response in the defined meaning of that term (2). In other words, the dry area remained completely devoid of sweat even when it was under the influence of lanolin. This absence of sweating on the inuncted part of the pad

* M. Dünner (Influence of Physical Factors [Pressure and Temperature] on Excretion of Sebum in Man, Dermatologica *93:* 249, 1946 and also E. O. Butcher and J. P. Parnell (The Distribution and Factors Influencing the Amount of Sebum on the Skin of the Forehead, J. Invest. Dermat. *10:* 31 [Jan.] 1948) have shown that sebum production increases with rise in local skin temperature.

area (which, incidentally, resembled true post-miliarial anhidrosis in that it was free of any obvious clinical sign of acute inflammation at the time of testing*)

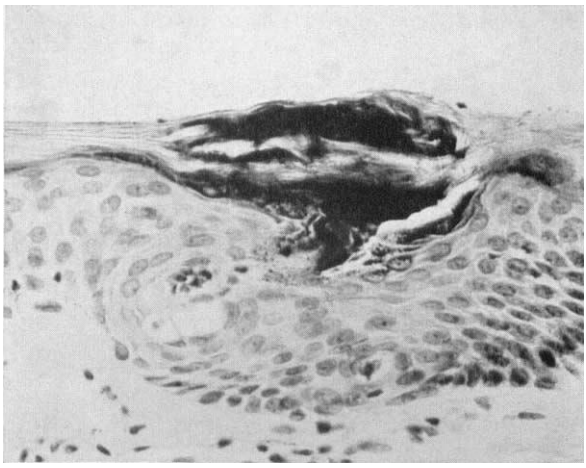


PLATE 15. A pore from pad-covered skin showing some increase in the volume of poral keratin. This appearance may represent edema. See Text. (H. and B. S. × 400.)

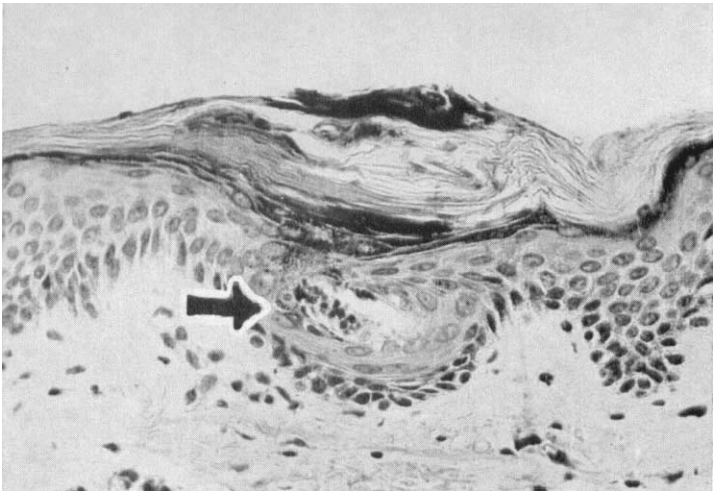


PLATE 16. Similar evidence of simple poral edema; notable however are the neutrophils in the deeper duct as indicated by an arrow (H. and B. S. × 400.)

suggests that the anhidrosis was not due to lipoid deprivation. For if it were so due, one might expect the sweat to have come through to the surface just as it does in post-miliarial anhidrosis under similar conditions.

* This point is made because little or no facilitation of visible sweating takes place when lipoid is smeared on skin which is diffusely involved by miliaria in its *acute inflammatory* stage (2).

The application of lipoid thus tends to confirm, but does not prove, the initial suspicion that this "envelopmental" type of anhidrosis is not due to simple lipoid deprivation and that it has to be explained in some other way.

The histologic changes in the pores do not support or exclude the influence of a lipoid-deficiency factor.

(ii) *Edema of the Stratum Corneum:* The second hypothesis harks back to Pollitzer (12) and is that the keratin of the stratum corneum swells through imbibition and so blocks the mouths of the ducts. Pollitzer, who spoke of natural miliaria, went further and proposed that the keratin swelled because it was not protected by sufficient lipoid. However, no objective evidence is at present available to support Pollitzer's claims, suggestive though they are. Keratin can im-

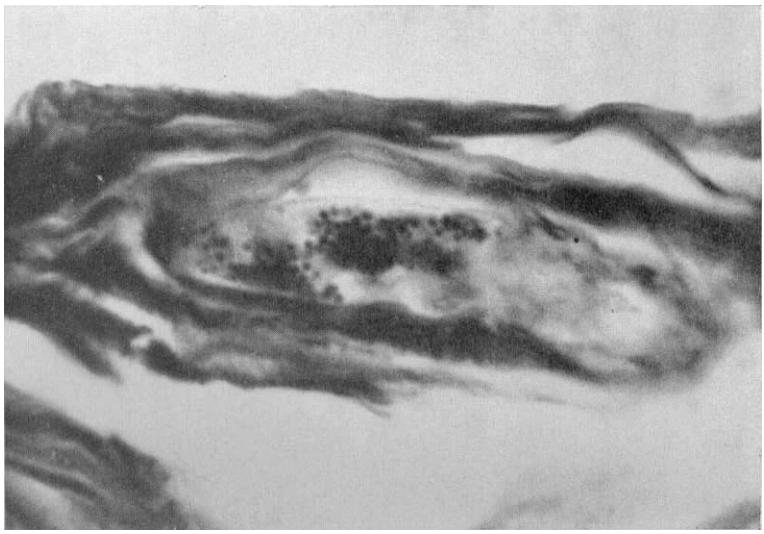


PLATE 17. A keratin ring from pad-covered skin. Numerous Gram-positive cocci are present. (Gram. × 1800.)

bibe water as is best seen in the hands when they are subjected to prolonged immersion (31) especially in alkaline media; but the vital point that such swelling obstructs the pores for long or even short periods remains yet to be proved. In the present experiments, poral edema does not adequately explain why the anhidrosis should persist for several days after the pads are removed and the keratin is allowed to dry out.

Recently I have found that when a moist solid vehicle (nutrient agar) containing Merthiolate (Lilly) is kept in contact with normal skin for a few days, no miliaria rubra resulted providing bacterial growth was successfully inhibited. Sometimes crystalline vesicles arose like those figured by Shelley *et al.* (4). As noted in Part 1 of this paper, the exact significance of such a crystalline response is not certain. For example, the crystallina resulting from covering the skin could be chiefly due to the presence on the skin surface of an accumulated, loose

lamina of the stratum corneum which, but for the protection afforded by the experiment, would have already been shed in the natural process of desquamation. Therefore the presence of crystalline vesicles does not prove beyond doubt the existence of a definitive or drastic degree of poral closure. Indeed it would not be difficult to support the reverse contention, that crystalline blockage of the pores is of its nature superficial, feeble and inconsequential.

To turn to the relevant histologic findings, Plates 15 and 16 are from the same biopsy as Plates 12 to 14 and give the typical appearances of those pores which might be considered as blocked by simple edema. The keratin in the pore areas seems increased in bulk and no bacteria or neutrophils* are identifiable within

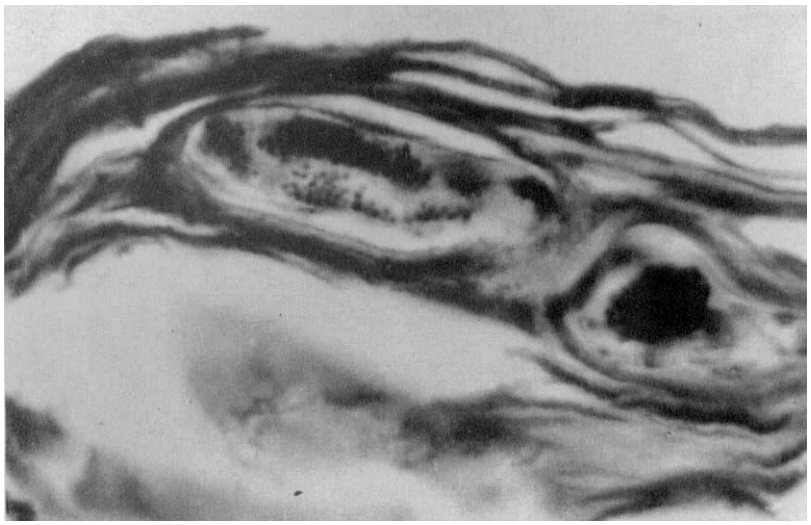


PLATE 18. An adjacent section of the same pore as shown in Plate 17. The ring, cut across in two places, is occupied by Gram-positive cocci. A single large clump of organisms almost fills the lumen on the right. (Gram. × 1800.)

it. Since there is no clear evidence of recent new formation of keratin (parakeratosis) the changes could conceivably be accounted for by edema alone. Nevertheless, despite such appearances, the evidence at present available does not justify a definite decision as to whether mere edema can initiate and maintain a blockage of the pores. It is significant that Pollitzer, when discussing natural miliaria, called in lipoid-depletion as an essential part of his concept.

In dealing with edema, one possible correlation should be kept in mind. It is that a temporary water-logging or maceration (Sulzberger and Zimmerman (9)) of the pore, whether or not it produces closure, may pave the way for infection by staphylococci and thus a prolonged, definitive closure (miliaria rubra or bullous impetigo). In this connection Arnold (32) has shown that edema of the stratum corneum causes an increase in the endogenous bacterial flora of the skin.

* However there are clumps of neutrophils in the *deeper* portions of the duct in Plate 16.

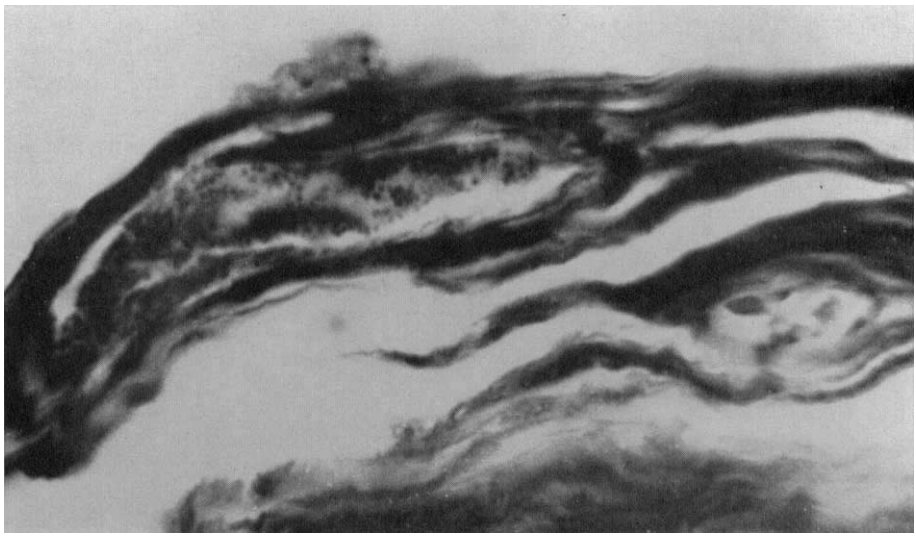


PLATE 19. Another section of the same pore. Gram-positive cocci present in the lumen on the left, neutrophils in the lumen on the right. (Gram. × 1800.)

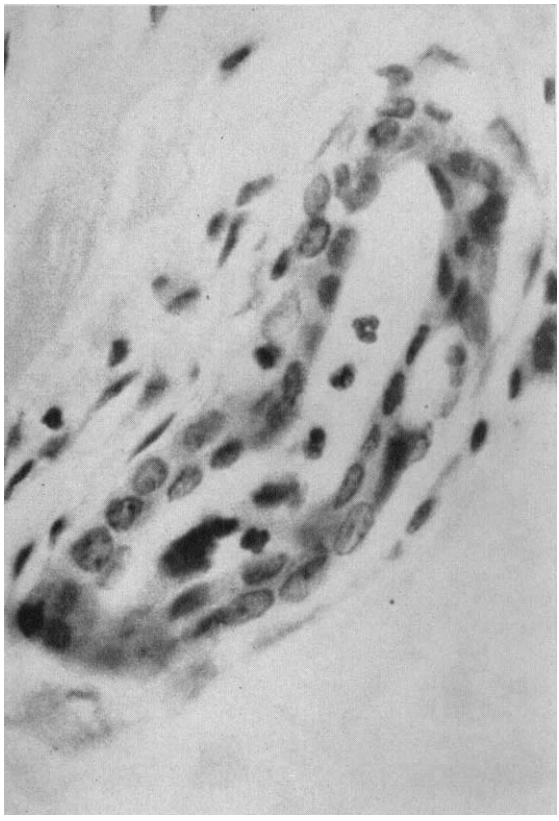


PLATE 20. A sweat duct from deep in the dermis of pad-covered skin. There are numerous neutrophils in the lumen. (H. and B. S. × 900.)

(iii) *Infection of the Pores under the Pad:* The biopsy from the anhidrotic area of skin contains some striking examples of infected pores.

Plate 17 shows a keratin ring containing dense clumps of Gram-positive cocci. Many neutrophils, (readily seen in the original) are out of focus in the background. Similar appearances of the same pore in an adjacent section are shown in Plate 18. One large clump of organisms appears to occlude the keratin ring almost entirely at one point. Plate 19 shows the same pore again and in this section neutrophils can be identified in an adjoining coil of the ring.

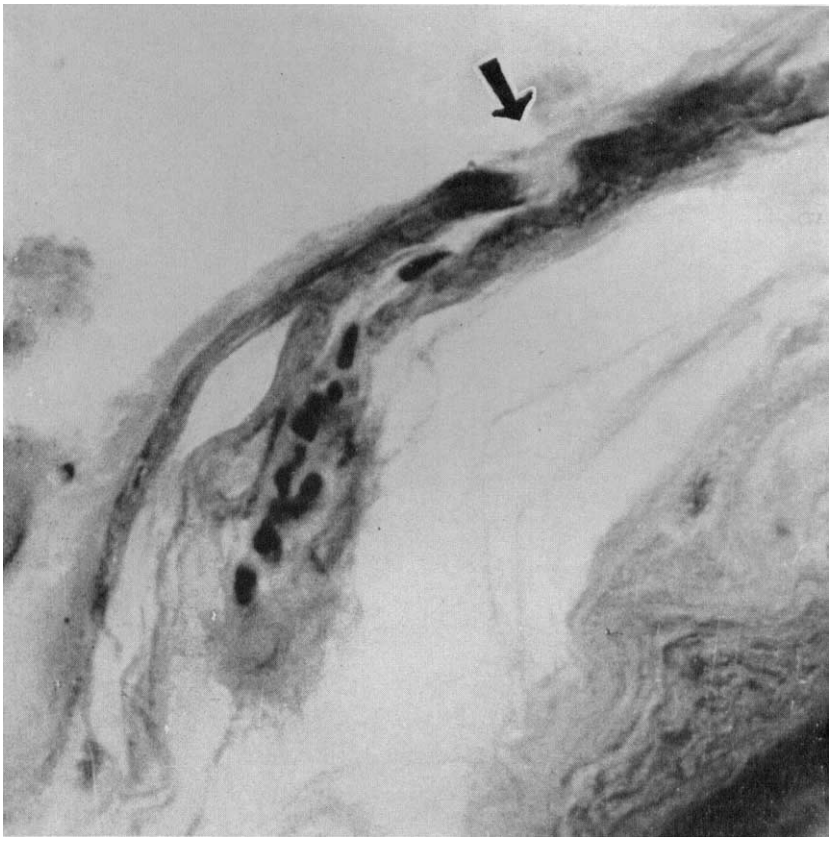


PLATE 21. Neutrophils present in a keratin ring as far as the mouth of the pore (shown by an arrow). The keratin ring has been partially detached from the skin surface (below, right). From pad-covered skin. (H. and B. S. × 1800.)

Further evidence as to the pyogenic character of the organisms within the ducts is furnished by the next two plates. The sweat duct in Plate 20 is from the middle of the dermis and contains conspicuous neutrophils. Another indication of ductal infection is the apparent migration of neutrophils right up to the very mouth of a pore in Plate 21. It therefore appears from the preceding five plates that Gram-positive cocci are infecting a proportion of the blocked pores and

that bacterial lesions identical with those often seen in natural miliaria (as in Plates 5 to 9) can be artificially produced by subjecting the skin to envelopment.

The question as to whether the infection is primary or secondary arises in regard to these effects of envelopment just as it does in miliaria itself. As the lesion shown in Plates 17 to 19 is presumably not more than 6 days old (and is possibly considerably less) it is a fair assumption to regard the infection as primary. If, for the sake of argument, the infection is dismissed as merely "secondary," the position still holds that cocci can be very readily induced to set up infection within the keratin rings by such simple means as the short application of a pad. Moreover, such infection is intimately associated with, if not directly causative of, intra-corneal obstruction and obstruction at that level is undoubtedly the essence of miliaria.

Presumably the application of a pad creates conditions of heat and humidity which favor the growth of resident but previously dormant pathogens. In other words, the pad is thought of as tipping the delicate host-organism balance in favor of the organisms. So high is the pathogenic carrier rate (19, 20, 21, 22) that these experiments clearly underline the role of cutaneous resistance and the ways in which it may be reduced.

In Summary, the final phases of the work done in the tropics gave rise to the following two working concepts:

(1) When the skin is kept covered, its intrinsic powers of resistance are reduced.

(2) The pores thereby become prone to staphylococcal infection with the result that a state of poral occlusion analogous to natural miliaria ensues.

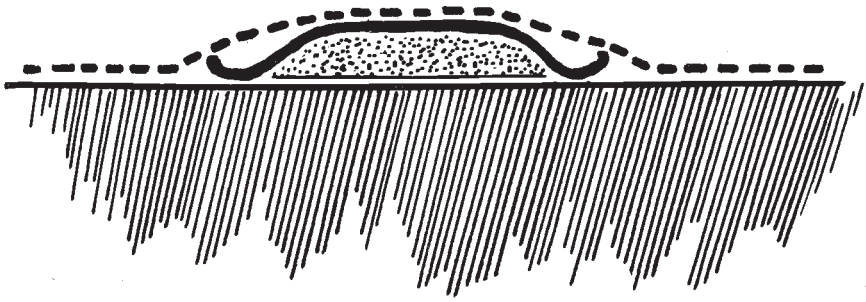
(c) *The production of staphylococcal poral infection in the subtropics by the application of solid nutrient media to the skin*

In a temperate climate, the responses arising out of the application of plain gauze pads to the skin are poorly defined and therefore difficult to interpret. Occlusion of the sweat pores, if it results at all, is slight and capricious even under the stress of prolonged envelopment. In explanation, it seems that in a cool climate the heat and humidity arising under a pad in contact with normal skin are simply not sufficient to give the resident flora a dominant advantage over the host. Therefore the post-war part of this investigation could not proceed in Sydney until the handicaps arising out of the milder environment were overcome.

After discarding various procedures, progress has lately been made by modifying the experimental conditions so as to ensure for the bacteria a really decisive advantage. This has been done by applying to the skin a solid nutrient medium rather than a plain gauze pad as previously used. The new procedure will be referred to as the "nutrient technic" and the present account will deal solely with its special application to staphylococcal infection, since, from the preceding pages, this is the aspect which most closely impinges on the problem of miliaria.

The earliest trials of the technic were carried out with ordinary nutrient agar and they revealed that a great number and variety of bacteria came to flourish on the interface between the skin and the medium. In fact, the richness of the resultant flora proved an embarrassment, especially as saprophytic bacteria of the *Bacillus subtilis* group often outgrew and suppressed any resident pathogens. Moreover cutaneous reactions were not obtained or were difficult to read until the medium employed was made strictly selective for staphylococci.

This aim was accomplished by applying the valuable observation of Hill and White (33) and of Koch (34) that staphylococci flourish on media containing high concentrations of sodium chloride whereas most other genera of bacteria do not; Chapman (35) and also Maitland and Martyn (36) have confirmed the observation. In this work I have employed ordinary nutrient agar (pH 7.5 or, later, 8.5) containing 8 per cent sodium chloride.



SKETCH 1. A cross section of agar-filled cup applied to the skin by means of adhesive tape

TECHNIC

General: Round cups, measuring some 3.0 cm. in diameter, 0.7 cm. in depth and of the shape shown in Sketch 1 were beaten from aluminum sheeting (gauge 20). After being autoclaved in Petri dishes the cups were filled to the brim with the melted salt agar, about 5.0 ml. being required for each.

Without any preparation of the skin, two agar-filled cups were applied to each volunteer under an ample amount of water-proof strapping. The "test" cup, applied to one side of the body, contained the 8 per cent salt agar, whereas the "control" cup, applied to the corresponding point on the opposite side of the body, contained the same salt medium plus an antibacterial substance (Merthiolate, Lilly) in sufficient concentration to prevent all bacterial growth. This control or sterile cup was used for the purpose of assessing non-infective effects such as might possibly have arisen as a result of increased local skin temperature, humidity, or substances (irritants, allergens) in the medium; the Merthiolate was added to melted medium in the proportion of one part per ten thousand parts.

In most experiments the cups were kept in position for 4 or 5 days; they were removed earlier if itching became prominent. On removal, the cutaneous responses were noted and, after making smears for Gram staining, direct sub-

cultures on horse blood agar plates were made from the surface of each cup. The plates were then incubated aerobically at 37°C for at least 24 hours. The actual contents of bullous lesions, if any arose, were also examined in a proportion of cases.

In a few instances where the responses were indefinite, two fresh cups were applied to the same areas for a further test period.

Initial procedure: isolation of a suitably pathogenic staphylococcus: Of the first five volunteers tested in this manner during a bout of humid weather, one (H.

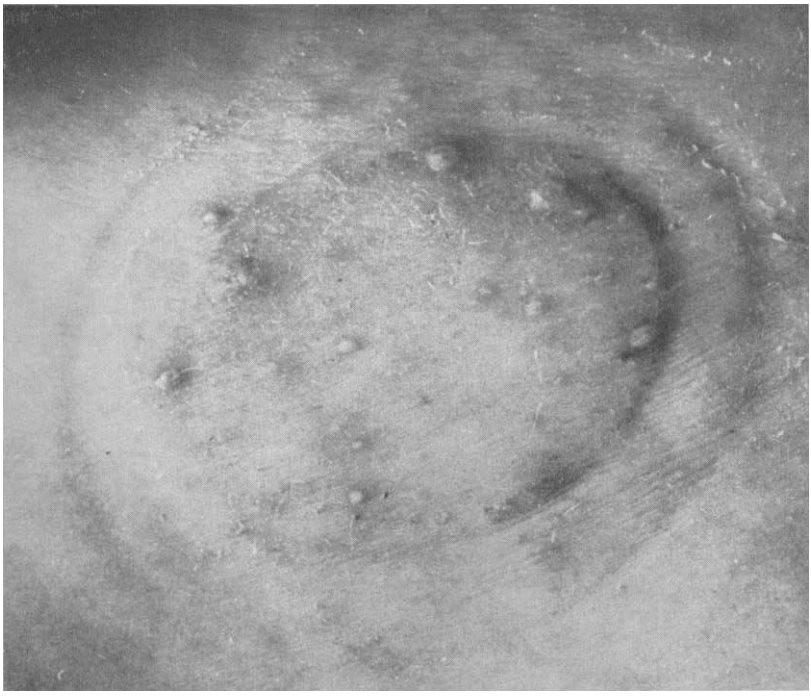


PLATE 22. Experimental Bullous Impetigo. *Staphylococcus aureus* was incubated on the skin in conjuction with salt agar. See Text. The inner circular indentation was produced by the cup holding the agar, the outer ring was produced by a larger empty cup applied simply to protect the rash.

F.) developed pruritus after two days and showed a typical diffuse bullous impetigo on the *test* area when the cups were removed on the fifth day (see Plate 22). Besides the pustules, small clear sweat vesicles were also evident. Cultural studies of the test cup surface and of the pustules demonstrated the presence of a profuse number of slightly hemolytic, coagulase-positive (37) staphylococci with colonies of pale golden color. There were also some colonies of "albus" staphylococci but no other genera of aerobic bacteria.

The rash on the test area of H. F. rapidly healed but was associated by local complete anhidrosis which lasted three weeks.

As regards the 4 volunteers who showed no rash on the *test* side, only "albus" coagulase-negative staphylococci were isolated from their respective test cups. No obvious rash or anhidrosis was produced on the *control* (sterile) side in any of the five subjects.

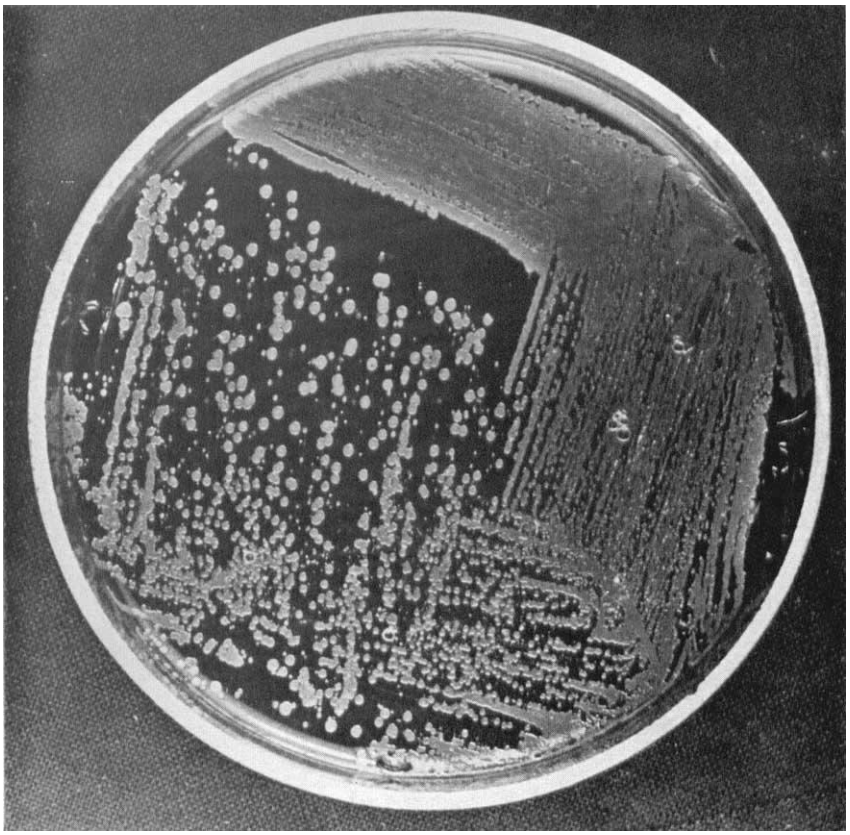


PLATE 22a. This picture illustrates that only staphylococci grow when salt agar is applied to the skin. This typical subculture was made from the surface of a test cup at the end of its period of application and consists of *Staph. aureus* (large colonies) and *Staph. albus* (small colonies).

Final Procedure: Because it had produced a poral rash (bullous impetigo), the staphylococcus isolated from H. F. (staphylococcus "H. F.") was employed in the remaining experiments, a loopful of broth culture being inoculated over the surface of all test cups just prior to their application.

RESULTS

In the summer of 1948–9 at Prince Henry Hospital, I applied the new technic in 24 separate experiments on 9 staff volunteers, the staphylococcus "H. F." being inoculated on the test cup. Providing the weather was warm enough,

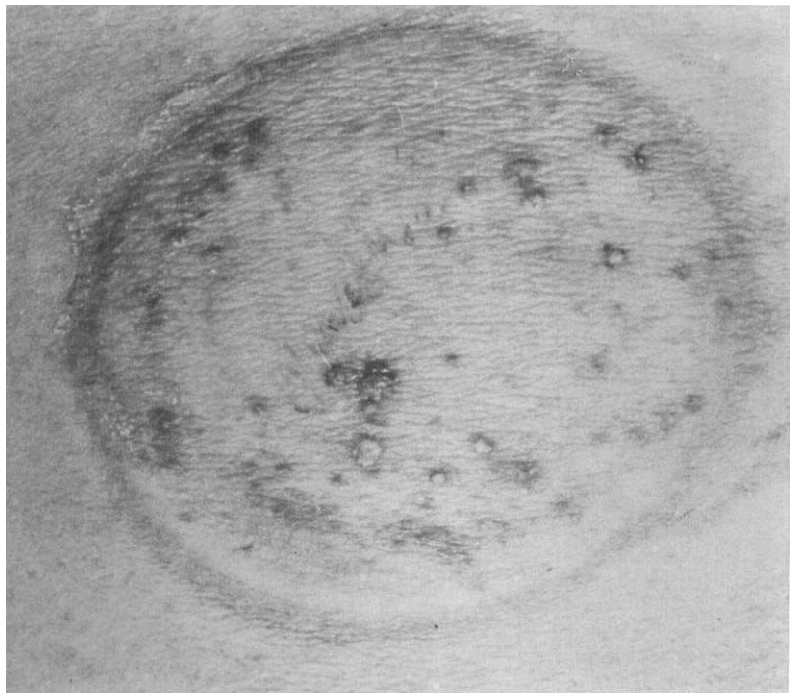


PLATE 23. Another example of experimental bullous impetigo. Towards the edge the rash has more resemblance to miliaria.

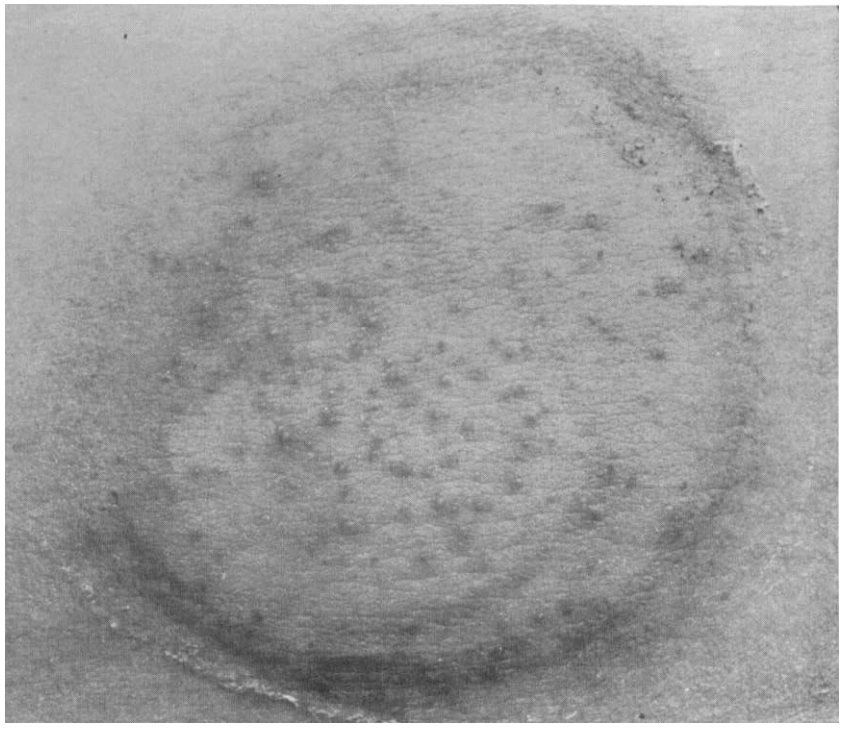


PLATE 24. A response regarded as typical miliaria rubra

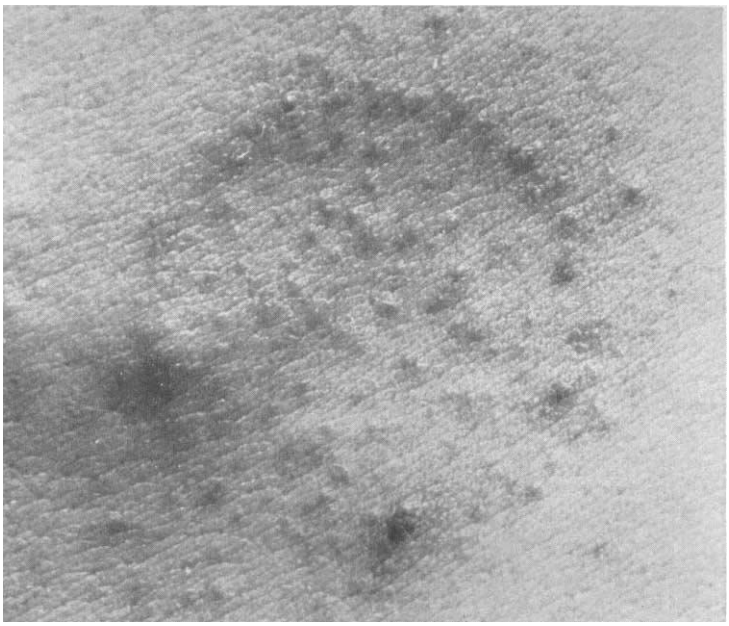


PLATE 25. A mixed response showing a few miliarial vesicles but mostly Bockhart's impetigo. On the left edge is an early furuncle which later aborted.

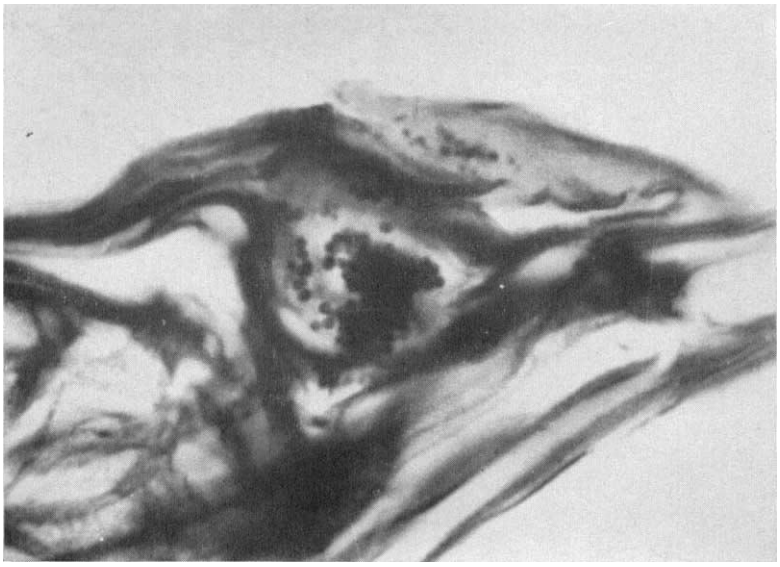


PLATE 26. Numerous staphylococci in a pore. An experimental lesion. (H. and B. S. × 1800.)

bullous impetigo and miliaria were produced with regularity on the *test* side. Details of the results together with some further comments on them are given in Table 1.

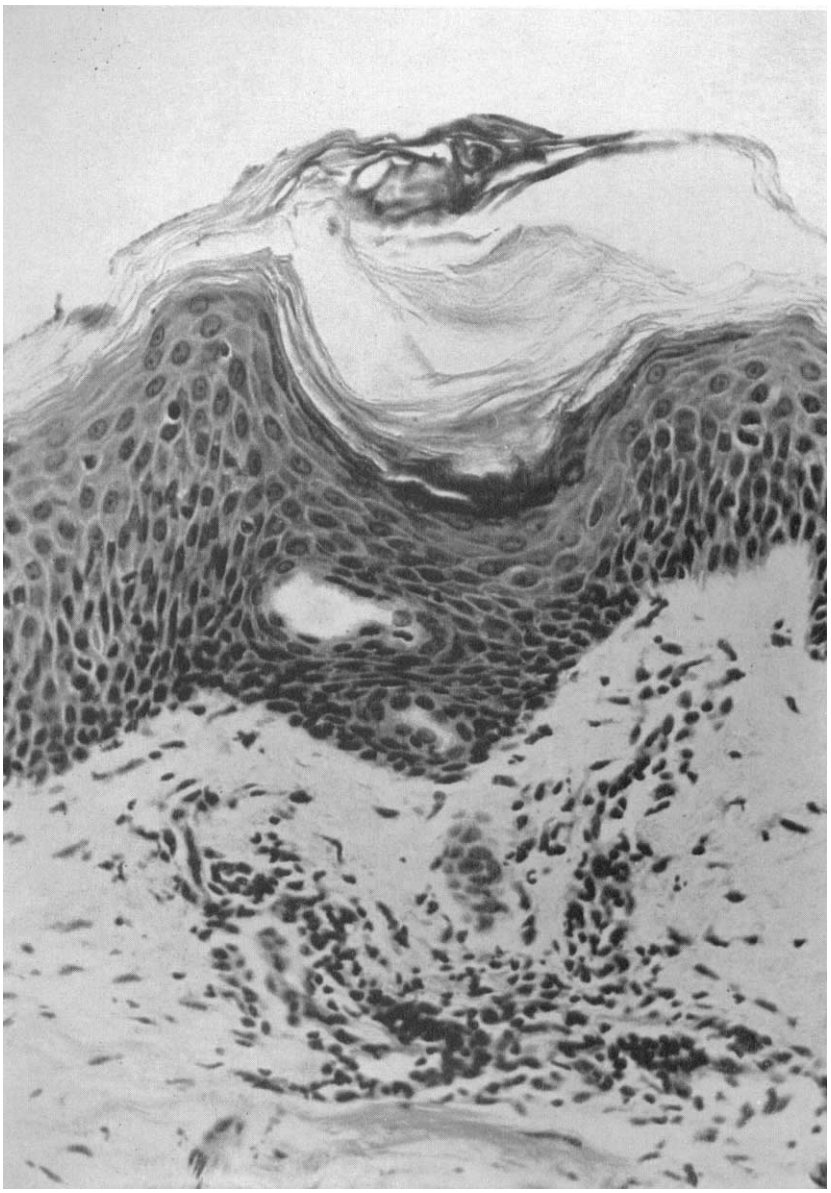


PLATE 27. The whole of the lesion of Plate 26. Notable are the pore on the surface, the clear sweat vesicle beneath it and the dilated sweat duct deeper in the malpighian layer, Numerous lymphocytes and a few neutrophils form a collar around the dermal duct. (H. and B. S. × 400.)

In most cases, as shown in Plate 23, miliarial and bullous lesions were present together. The results as a whole emphasized the close affinity of miliaria and impetigo; indeed it was often impossible to decide whether a particular lesion

TABLE 1

Experimental production of poral rashes (bullous impetigo and miliaria)

Details of Twenty-Four Experiments on Skin of Volunteers. Application of *Staphylococcus Aureus* ("H.F.") in Conjunction with Salt Agar Cups (see Text)

RESPONSE OBTAINED (NUMBER OF TIMES)	TEST (APPLICATION OF CUP OF 8% SALT AGAR INOCULATED WITH STAPHYLOCOCCUS "H. F.").		CONTROL (SIMULTANEOUS APPLICATION OF CUP OF 8% SALT AGAR CONTAINING 1/10,000 MERTHIOLATE).		
	Clinical Result on Cup Area	Result of Direct Subculture from Test Cup Surface	Clinical Result on Cup Area	Result of Direct Subculture from Control Cup Surface	Remarks
8	Bullous Impetigo and/ or Miliaria Rubra	Profuse *Staph. aureus* (*Staph. albus* also present)	Nil except for probable maceration of stratum corneum	Sterile	—
7	Bullous Impetigo and/ or Miliaria Rubra	Profuse *Staph. aureus* (*Staph. albus* also present)	Nil except for probable maceration of stratum corneum	*Staph. albus* only	Sterilization on control side not complete
3	Bullous Impetigo and/ or miliaria Rubra	Profuse *Staph. aureus* (*Staph. albus* also present)	Crystallina	Sterile	—
1	Bullous Impetigo and/ or Miliaria Rubra	Profuse *Staph. aureus* (*Staph. albus* also present)	Crystallina	*Staph. albus* only	Sterilization on control side not complete
1	Bullous Impetigo and/ or Miliaria Rubra	Profuse *Staph. aureus* (*Staph. albus* also present)	Fine macular erythema (? nature)	*Staph. albus* only	Sterilization on control side not complete
3	Bullous Impetigo and/ or Miliaria Rubra	Profuse *Staph. aureus* (*Staph. albus* also present)	Bullous Impetigo and/ or Miliaria Rubra	*Staph. aureus* and *Staph. albus*	1. Sterlization on control side not complete 2. Control cup contaminated by *Staph. aureus* (subjects were possibly pathogenic skin carriers)

1	One Bulla only	Scanty *Staph. aureus* (*Staph. albus* profuse)	One Pustule only, (? nature)	*Staph. albus* only	1. Result indecisive 2. Subject was a poor sweater 3. Sterilization on control side not complete

Total = 24.

Notes. Duration of each experiment, 4–5 days.

For reasons given in the text, bullous impetigo and miliaria rubra have not been discriminated in the table. Had a thermal cabinet as used by Shelley *et al.* (4) been available, crystallina would possibly have been observed more often on the control side.

Follicular lesions are not included in the table.

By *Staph. aureus* is meant a pigmented, coagulase-positive, haemolytic staphylococcus. By *Staph. albus* is meant a white, coagulase-negative, non-haemolytic staphylococcus.

was of miliarial or *early* bullous nature. Thus there was no way of making a hard and fast distinction between the two states and they are grouped together

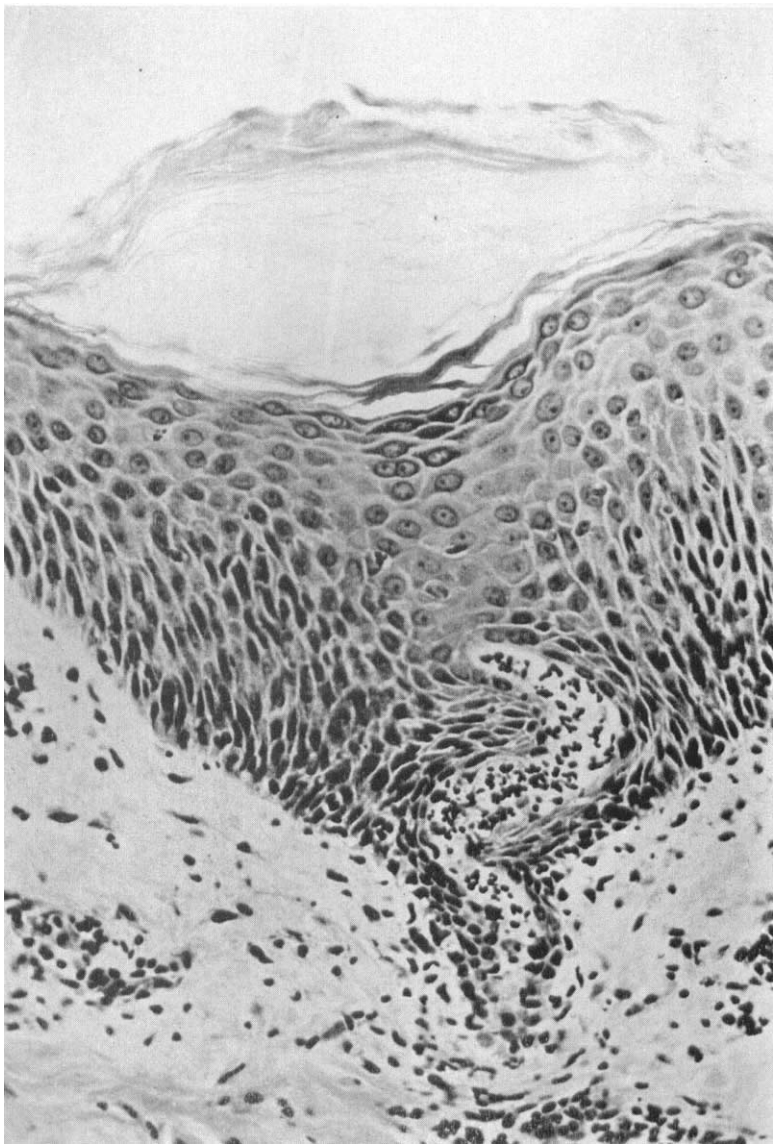


PLATE 28. Another experimental sweat vesicle. Very many neutrophils crowd the deeper duct and are apparently on their way to the vesicle on the surface. (H. and B. S. × 400.)

in the second column of Table 1. Whether this consolidation of results is completely justified merits further research.

Plate 24 shows another response from the series, in this case a typical miliaria rubra. Some miliarial vesicles are also seen in the response illustrated in Plate 25.

Hemolytic coagulase-positive staphylococci were recovered in profusion from the test cups at the end of all experiments. No phage typing was done in any

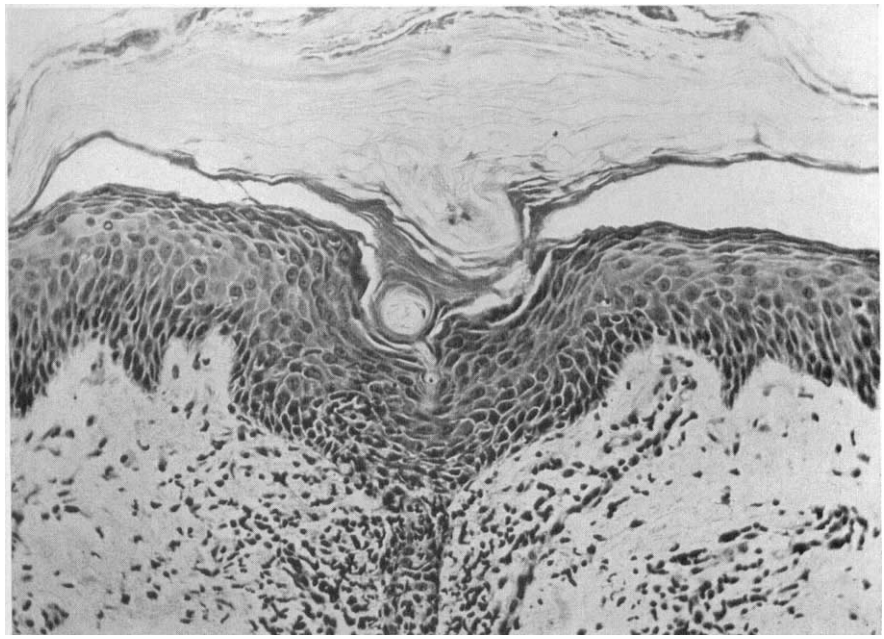


PLATE 29. A large experimental bullous vesicle; the poral nature of the lesion is again obvious. (H. and B. S. × 330.)

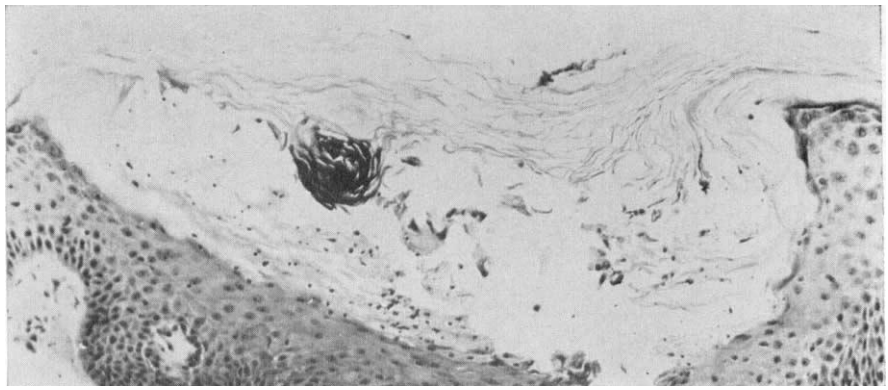


PLATE 30. A still larger and more mature bullous vesicle containing pus cells. The closed keratin ring of the associated pore appears as a very dark round mass. (H. and B. S. × 200.)

experiment to determine whether or not staphylococcus "H. F." had been contaminated with pathogenic strains derived from (resident on) the skin of the particular subject. In other words, the observations are held to incriminate

pathogenic staphylococci as a group rather than any special "strain" of the organism.

Where bullous impetigo and/or miliaria arose on the *control* side, *Staphylococcus aureus* was found to be present and to have grown despite the Merthiolate.

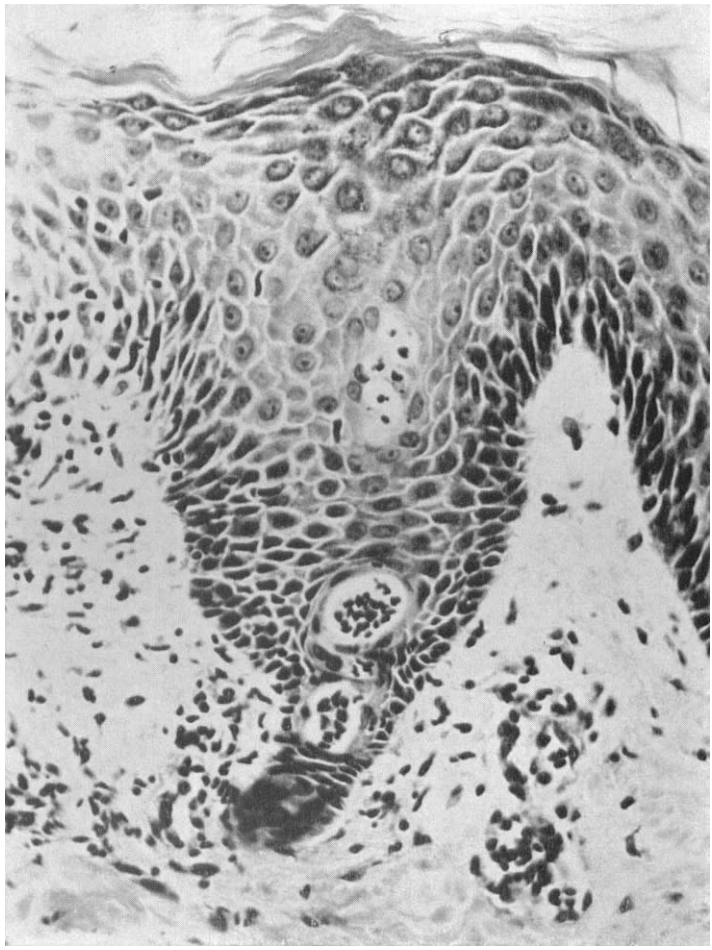


PLATE 31. A miliaria-like lesion in experimental staphylococcal infection. There are deep distension of the duct and periductal engorgement and inflammatory infiltration, Neutrophils are more numerous within the duct than is common in natural miliaria. (H. and B. S. × 400.)

Where the control side remained free of *Staphylococcus aureus*, crystallina was the only definite response; it was detected by ordinary clinical examination in four instances. A thermal cabinet such as described by Shelley *et al.* (4) was not available to study the reactions of the control side in full detail as regards possible minor degrees of crystallina and anhidrosis.

The Histopathology of Experimental Staphylococcal Poral Infection: This account

is based on over 1,000 serial sections made from 2 biopsies. The rashes concerned showed a mixture of bullous and miliarial lesions and had been produced on the same subject (J. P. O'B.) on different occasions.

Plate 26 is an example of the many infected sweat pores present in the biopsies. It should be compared with infected pores from natural miliaria rubra (Plates 5 to 9).

Plate 27 gives a low power view of the whole of the lesion of Plate 26. It shows a superficial early (clear) sweat vesicle and also a dilatation of the deeper duct.

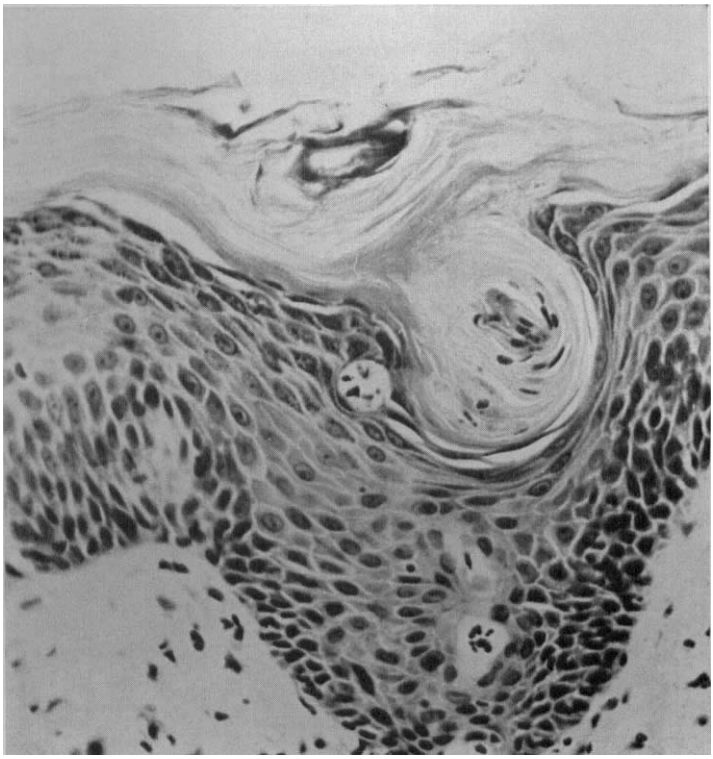


PLATE 32. A sweat pore contains a parakeratotic plug as a result of experimental staphylococcal infection. (H. and B. S. × 400.)

A later though otherwise similar lesion is illustrated in Plate 28. The deeper portion of the sweat duct is occupied by numerous neutrophils which are apparently on their way to the superficial vesicle.

A large and definite bulla is shown in Plate 29. Neutrophilic invasion of the deep sweat duct is again striking.

A still larger bulla containing some pus cells is given in Plate 30. Evidence of the associated sweat pore can still be made out.

Plates 31 and 32 are pictures which closely parallel the appearances found in natural miliaria (2, 9). In Plate 31 is a deep distension which can be regarded as

a prelude to duct rupture and thus full-blown miliaria. In Plate 32 is a large parakeratotic plug identical in appearance with those found in natural miliaria.

Follicular Changes: Various pathologic changes in the pilosebaceous follicles can be produced by the new technic. Bockhart's impetigo, acneform lesions and abortive furunculosis deserve special mention (see Plate 25). A fuller analysis of these responses and of their probable bearing on acne will be the subjects of a future paper.

DISCUSSION

When used in conjunction with a particular strain of *Staphylococcus aureus*, the nutrient technic described in this paper can be used to produce poral rashes (bullous impetigo, miliaria) in a temperate climate during summer.

The results obtained lend further support for the hypothesis that bullous impetigo and miliaria rubra are closely related diseases. The nature of the response in a given case may be determined by the interplay of the host's resistance and the invader's virulence.

This work also suggests that, even in a cool climate, many impetiginous rashes are initially poral in origin. Whether or not this also applies to staphylococcal impetigo contagiosa is a matter for further research.

Attempts by previous workers to produce impetigo have been only partly successful and some form of injury to the skin has usually been found necessary. For instance Bigger and Hodgson (38) could not produce impetigo contagiosa by any of some sixteen different methods, whereas Sheehan and Ferguson (39) were successful only when the skin was vigorously scarified by a needle. Even then, fully developed impetigo occurred in only a quarter of the experiments.

The present work with occlusive dressings and agar cups gives information on contributory factors in impetigo and miliaria. The principal ones appear to be increased temperature and humidity of the stratum corneum. However, these factors acting alone seem unable to produce a really significant or persistent degree of poral occlusion.

Contrary to the deductions drawn from earlier work (2), a severe degree of lipoid depletion of the stratum corneum is probably not prerequisite. However, as a possible contributory factor, lipoid depletion could theoretically have a direct occlusive (? constrictive (2)) effect on the pores or else it could act by favoring both edema (32) and infection (40). It must still be admitted that there are some ichthyoid states in which lipoid depletion appears to be the primary factor responsible for maintaining poral closure.

Apart from miliaria, the nutrient technic should be useful in other fields of investigation. For instance, it should prove to be the most sensitive method yet available for detecting the carriage of pathogenic staphylococci on the skin.

SUMMARY OF PART II

Experiments wherein the skin was covered by plain gauze pads originally suggested that staphylococcal infection of the sweat pores is an important causative factor in miliaria rubra.

This suggestion was later supported by a new technic in which a special nutrient agar was inoculated with *Straphylococcus aureus* and then applied to the skin. Biopsies of skin so treated show lesions which appear identical with those of the natural disease.

When this evidence is considered in conjunction with the knowledge that Gram-positive cocci are identifiable with considerable regularity in the pores in the very earliest lesions of natural miliaria, the evidence supporting primary staphylococcal infection becomes impressive. *Staphylococcus aureus* of one or more strains is probably the organism responsible.

The belief that bullous impetigo is of a similar infective origin is confirmed.

Edema and increased temperature of the stratum corneum appear as contributory factors in both miliaria rubra and impetigo. Despite earlier views (2), lipoid depletion is probably not the sole or direct cause of miliaria as seen in the tropics. Nevertheless a lipoid depletion is probably the direct cause of poral closure in many ichthyotic skins.

Where infection is absent, edema and increased temperature are together capable of producing only a trivial and transient degree of poral closure as manifested in crystallina.

(The References will be given at the end of Part IV).